



## REPLACEMENT SHEET

Heller Ehrman White &amp; McAuliffe, LLP

Sheet 1 of 13

Title: Positional Sequencing by Hybridization.  
Applicants: Cantor *et al.* Attorney Docket No. 25491-2401G  
U.S. Serial No.: 09/030,571 Filing Date: February 24, 1998

FIG. 1

NUCLEIC ACID  
STRUCTURECALCULATED  $T_m$  ( $^{\circ}\text{C}$ , AVERAGE BASE COMPOSITION) $n = 8 \quad 7 \quad 6 \quad 5$ 

<u>      </u>	38	33	25	15
<u>      </u>	33	25	15	3
<u>      </u>	25	15	3	-14
<u>      </u>	51	46	40	31
<u>      </u>	46	40	31	21
<u>      </u>	40	41	21	11



REPLACEMENT SHEET  
Heller Ehrman White & McAuliffe, LLP  
Sheet 2 of 13  
Title: Positional Sequencing by Hybridization.  
Applicants: Cantor et al. Attorney Docket No. 25491-2401G  
U.S. Serial No.: 09/030,571 Filing Date: February 24, 1998

FIG. 2A

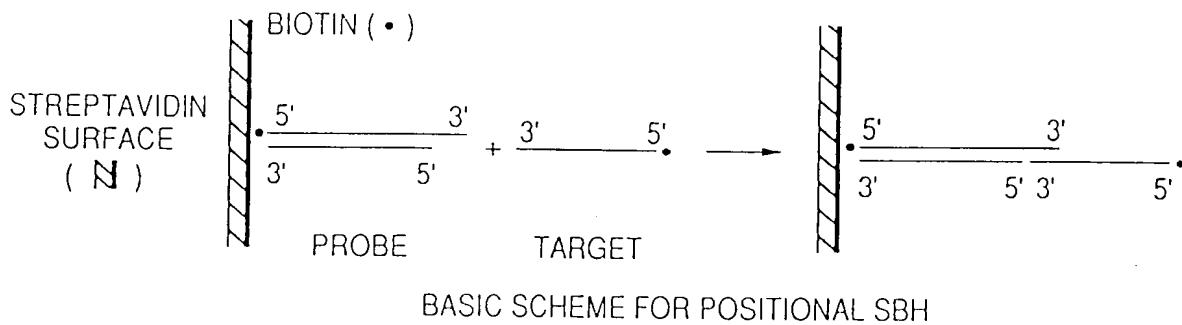
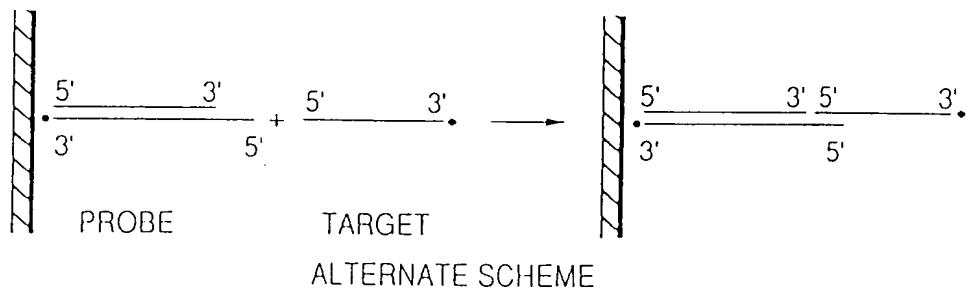


FIG. 2B





REPLACEMENT SHEET  
Heller Ehrman White & McAuliffe, LLP  
Sheet 3 of 13  
Title: Positional Sequencing by Hybridization.  
Applicants: Cantor *et al.* Attorney Docket No. 25491-2401G  
U.S. Serial No.: 09/030,571 Filing Date: February 24, 1998

FIG. 3A

LIGATION OF TARGET DNA WITH PROBE

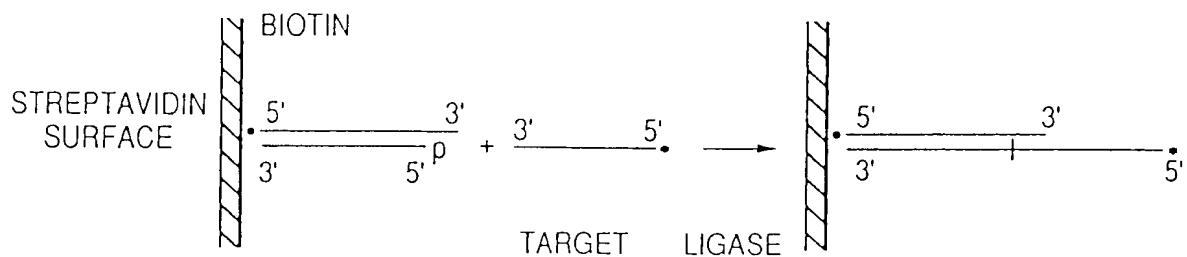
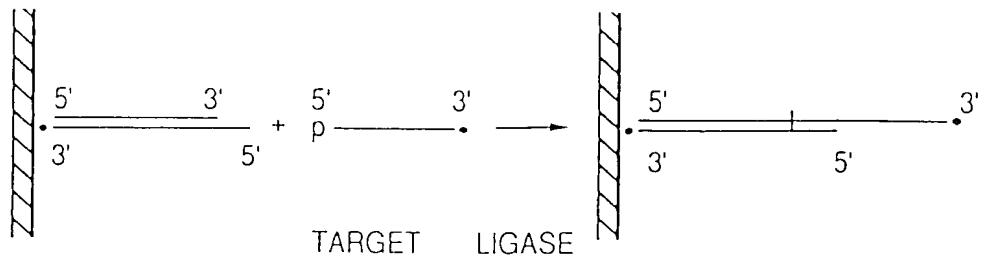


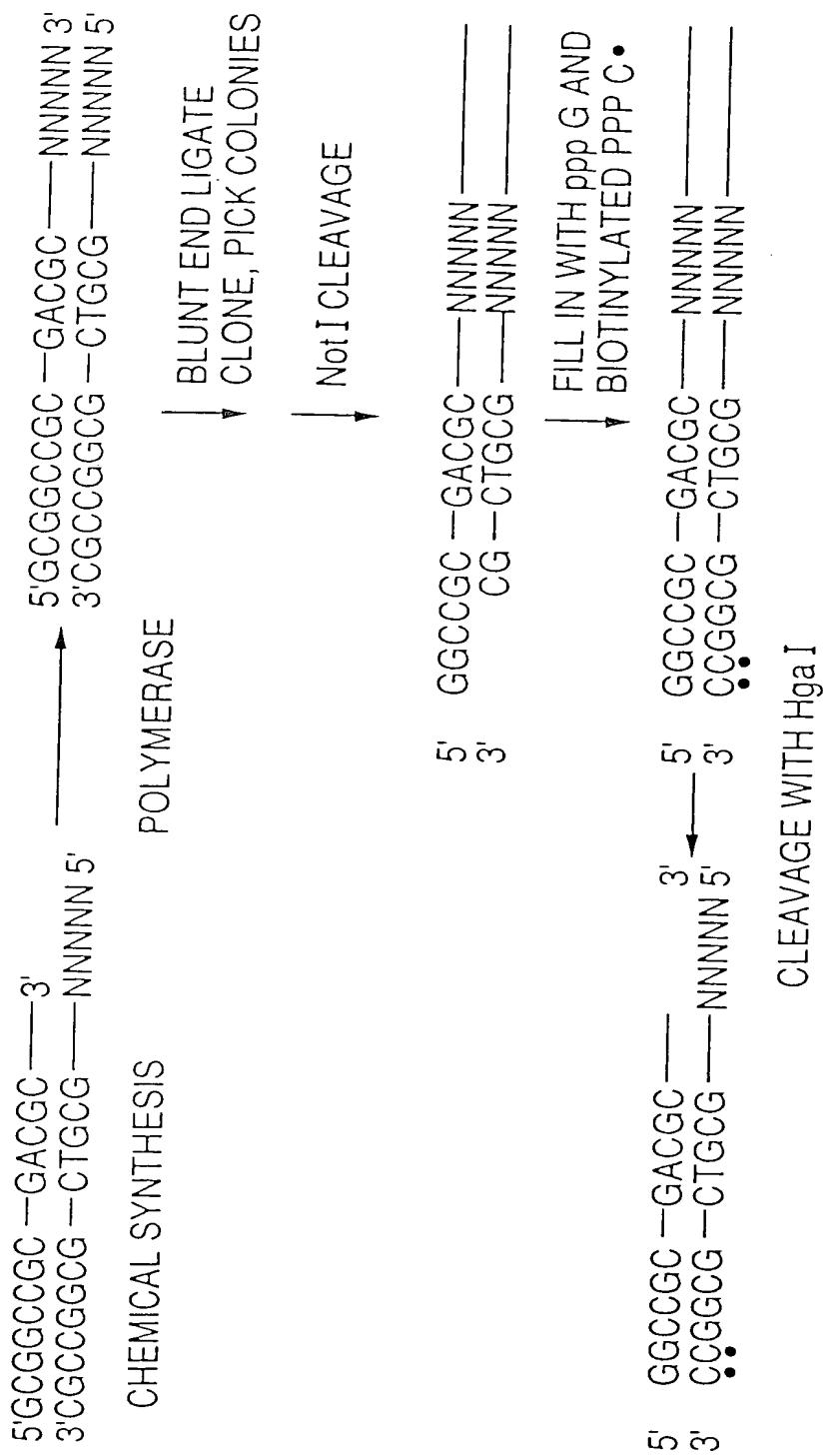
FIG. 3B





**REPLACEMENT SHEET**  
 Heller Ehrman White & McAuliffe, LLP  
 Sheet 4 of 13  
 Title: Positional Sequencing by Hybridization.  
 Applicants: Cantor *et al.* Attorney Docket No. 25491-2401G  
 U.S. Serial No.: 09/030,571 Filing Date: February 24, 1998

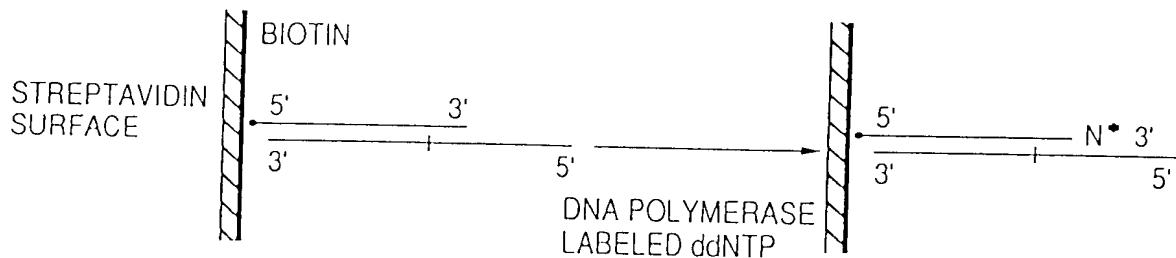
FIG. 4





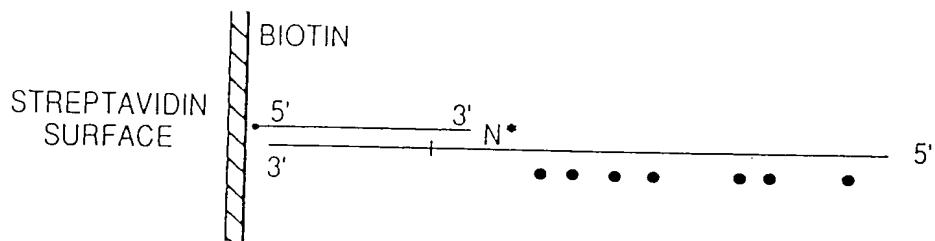
REPLACEMENT SHEET  
Heller Ehrman White & McAuliffe, LLP  
Sheet 5 of 13  
Title: Positional Sequencing by Hybridization.  
Applicants: Cantor *et al.* Attorney Docket No. 25491-2401G  
U.S. Serial No.: 09/030,571 Filing Date: February 24, 1998

FIG. 5



READING AN EXTRA TARGET BASE BY 3' EXTENSION OF THE PROBE

FIG. 7



POSITIONAL INFORMATION FROM RATIO OF INTERNAL LABEL (•) TO EXTENSION LABEL (••). A 5' LABEL COULD ALSO BE USED.

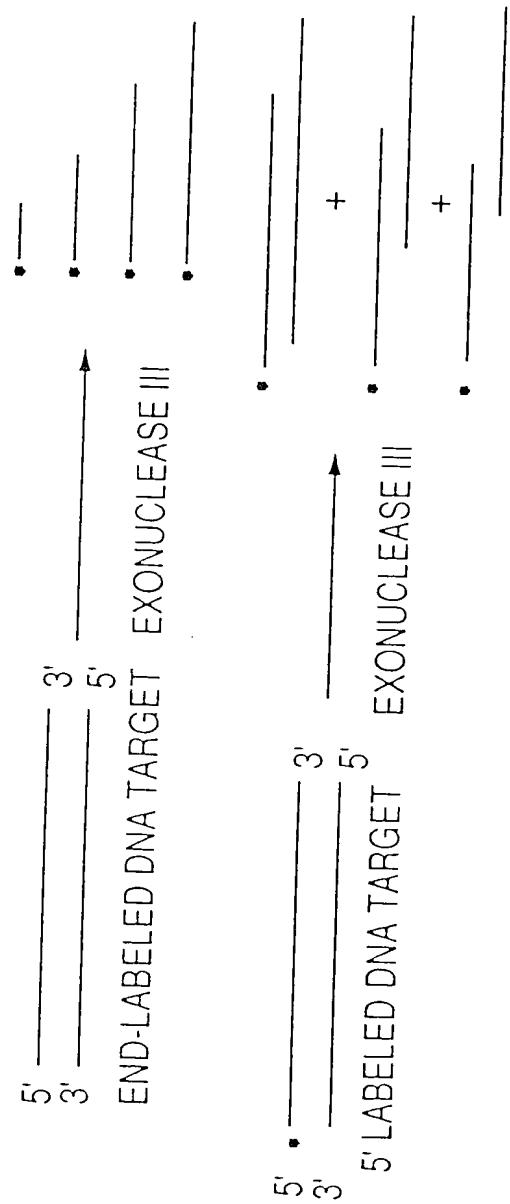


REPLACEMENT SHEET  
Heller Ehrman White & McAuliffe, LLP

Sheet 6 of 13

Title: Positional Sequencing by Hybridization.  
Applicants: Cantor *et al.* Attorney Docket No. 25491-2401G  
U.S. Serial No.: 09/030,571 Filing Date: February 24, 1998

FIG. 6



PREPARATION OF A NESTED SET OF DNAs



REPLACEMENT SHEET  
Heller Ehrman White & McAuliffe, LLP  
Sheet 7 of 13

**Title:** Positional Sequencing by Hybridization.  
**Applicants:** Cantor *et al.* **Attorney Docket No:** 25491-2401G  
**U.S. Serial No.:** 09/030,571 **Filing Date:** February 24, 1998

7 of 15

Sheet 7 of 13

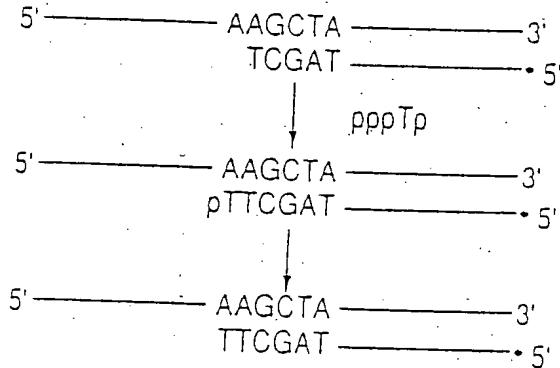
## Title: Positional Sequencing by Hybridization

Applicants: Cantor *et al.* Attorney Docket No. 25491-2401G

U.S. Serial No.: 09/030,571 Filing Date: February 24, 1998

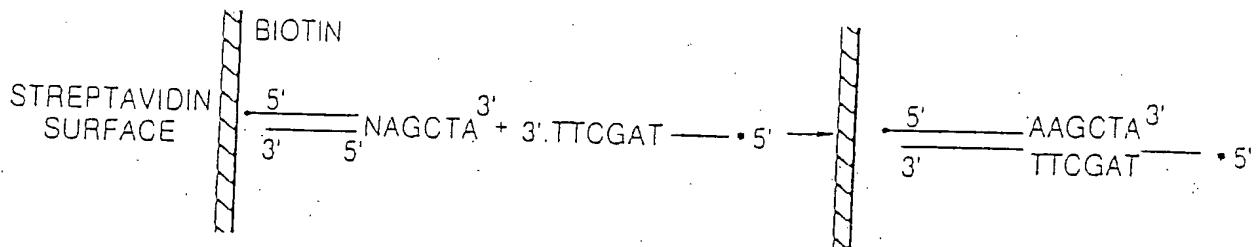
## READING AN EXTRA TARGET BASE

## READING AN EXTRA TARGET BASE



EXTENSION WILL FAIL WITH pppAp, pppGp, AND pppCp

#### A. 3' EXTENSION OF THE TARGET WITH A pppN<sub>p</sub> PRIOR TO PHOSPHATASE TREATMENT



## ARRAY WITH ONE DEGENERATE BASE

#### B. HYBRIDIZATION AND LIGATION



**REPLACEMENT SHEET**  
**Heller Ehrman White & McAuliffe, LLP**  
**Sheet 8 of 13**  
**Title: Positional Sequencing by Hybridization.**  
**Applicants: Cantor *et al.* Attorney Docket No. 25491-2401G**  
**U.S. Serial No.: 09/030,571 Filing Date: February 24, 1998**

**FIG. 9**

**LABEL INTENSITY**

CHAIN TERMINATOR: SEQUENCE	T	G	A
GGAAT	2A, 2G	—	2G
AAGGT	2A, 2G	2A	—
GAAGT	2A, 2G	—	1G
AGGAT	2A, 2G	1A	—
AGAGT	2A, 2G	1A	—
GAGAT	2A, 2G	—	1G
GGGAT	1A, 3G	—	3G
GGAGT	1A, 3G	—	2G
GAGGT	1A, 3G	—	1G
AGGGT	1A, 3G	1A	—
AAAGT	3A, 1G	3A	—
AAGAT	3A, 1G	2A	—
AGAAT	3A, 1G	1A	—
GAAAT	3A, 1G	—	1G
GGGGT	4G	—	1T, 4G*
AAAAT	4A	1T, 4*	—

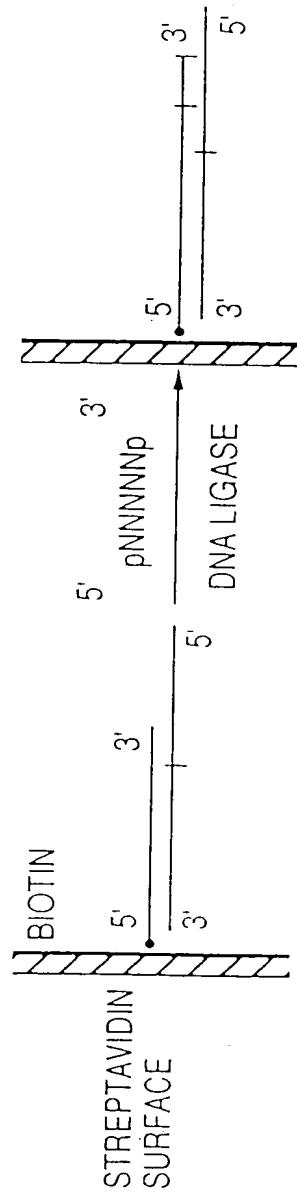
FOUR COLOR ANALYSIS OF SEQUENCE EXTENSION OF THE 3' END OF  
 THE PROBE. LABEL INTENSITY SHOWN DOES NOT INCLUDE CONTRIBUTION  
 FROM THE 3' TERMINATOR.

\* PLUS ADDITIONAL POSSIBLE RESIDUES



REPLACEMENT SHEET  
Heller Ehrman White & McAuliffe, LLP  
Sheet 9 of 13  
Title: Positional Sequencing by Hybridization.  
Applicants: Cantor *et al.* Attorney Docket No. 25491-2401G  
U.S. Serial No.: 09/030,571 Filing Date: February 24, 1998

FIG. 10

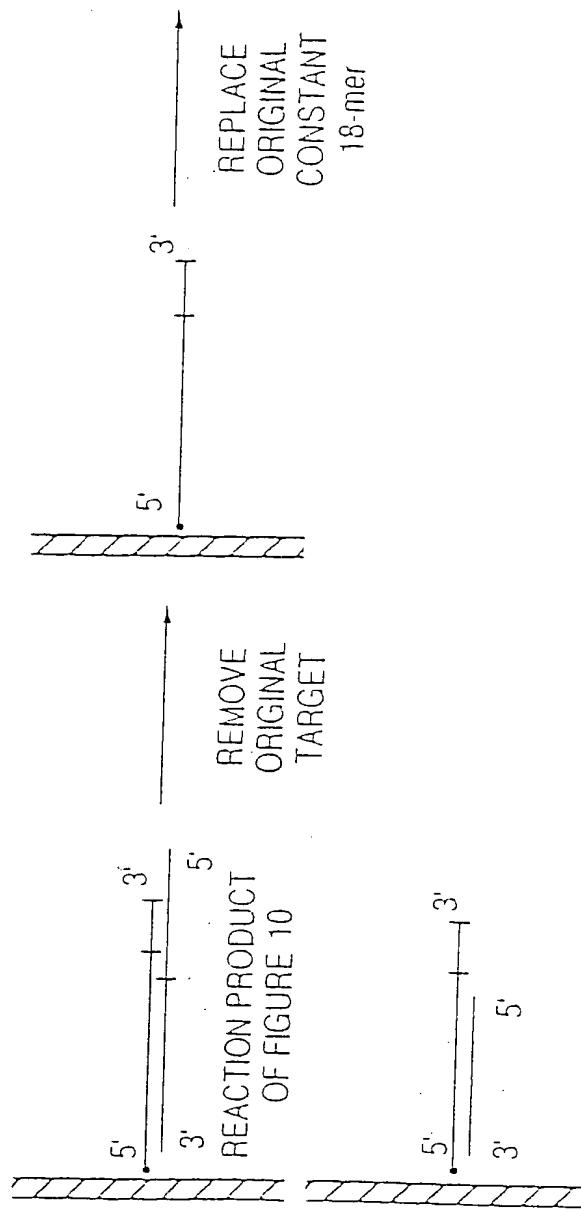


EXTENSION OF THE PROBE BY LIGATION OF A BLOCKED PENTANUCLEOTIDE.  
THE PENTANUCLEOTIDE IS 3'-BLOCKED TO PREVENT POLYMERIZATION.



REPLACEMENT SHEET  
Heller Ehrman White & McAuliffe, LLP  
Sheet 10 of 13  
Title: Positional Sequencing by Hybridization.  
Applicants: Cantor et al. Attorney Docket No. 25491-2401G  
U.S. Serial No.: 09/030,571 Filing Date: February 24, 1998

FIG. 11



PREPARATION OF A CUSTOMIZED PROBE CONTAINING A 10 bp SEQUENCE  
PRESENT IN THE ORIGINAL TARGET DNA



**FIG. 12**

**PROBE AND TARGET ANNEALED**

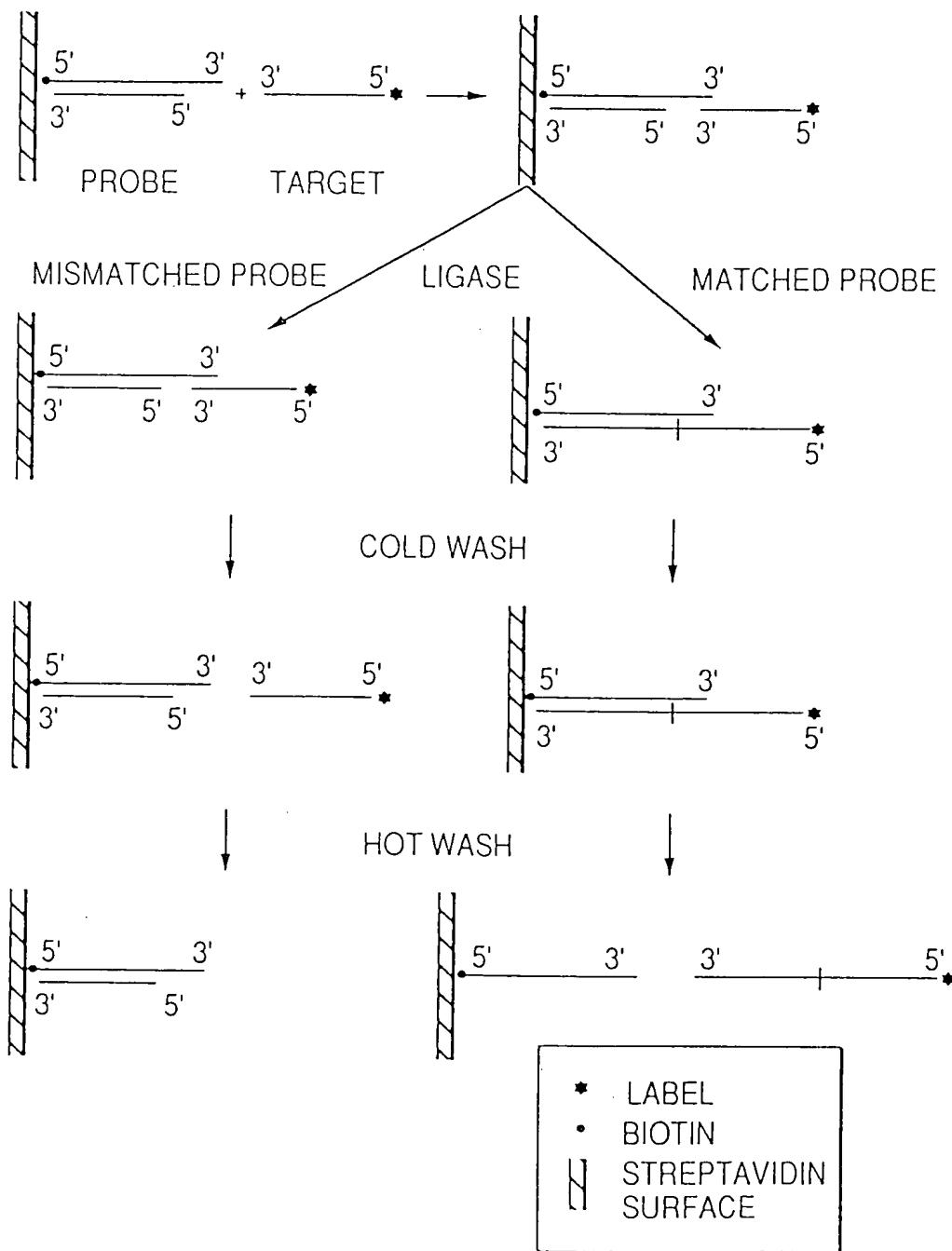
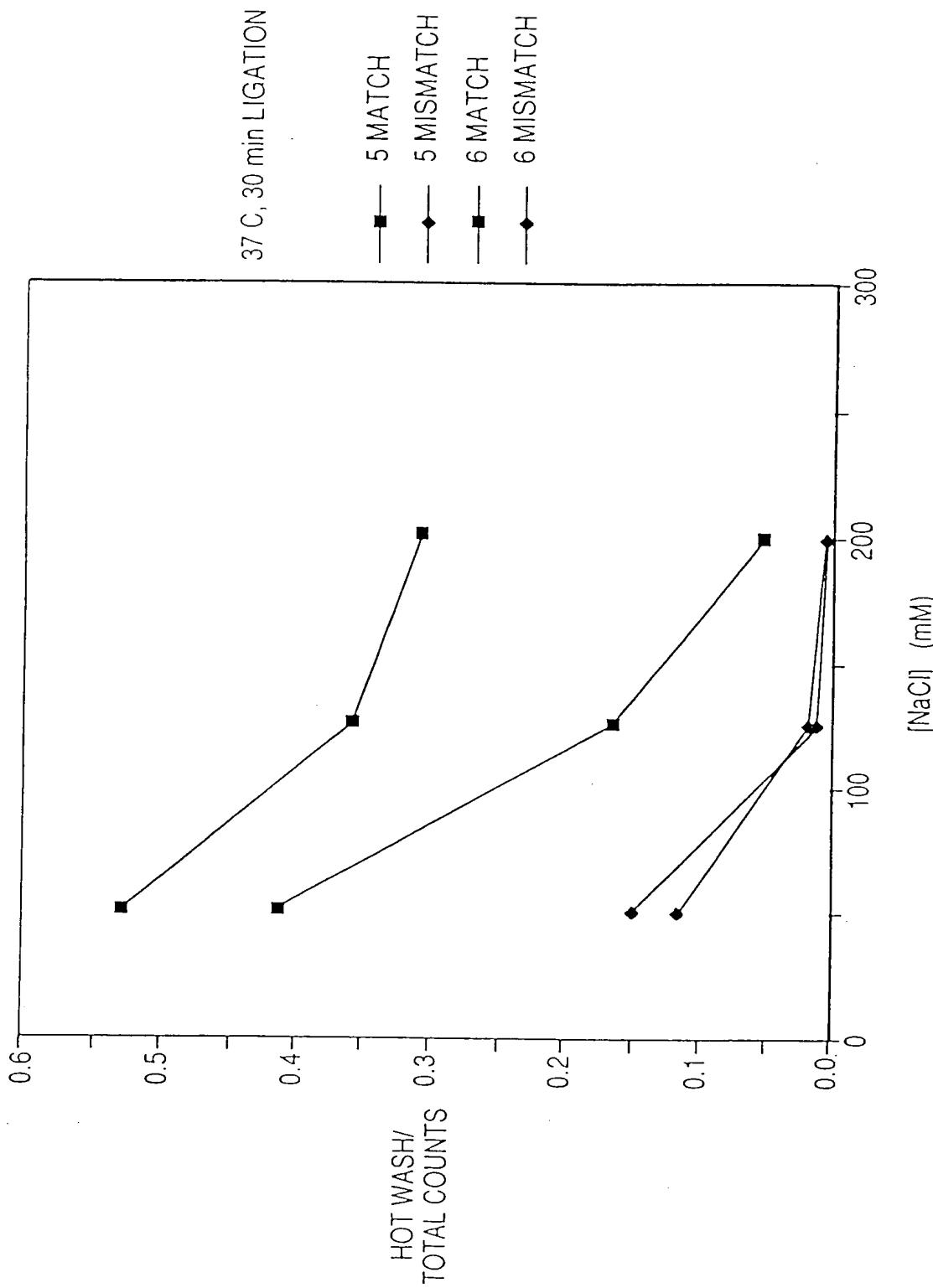




FIG. 13





**FIG. 14**

